

Genital abnormalities in white-tailed deer (*Odocoileus virginianus*) in west-central Montana : Pesticide exposure as a possible cause.

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Abstract : From spring, 1996, to early spring, 2000, accident-killed and injured white-tailed deer, *Odocoileus virginianus*, in the Bitterroot Valley of west-central, Montana, U.S.A., were collected and examined for genital abnormalities at the Bitterroot Wildlife Rehabilitation Center. Of the 254 male deer examined, 133 were fawns aged 3 months to 1 yr, 29 were 1 to 1½ yrs of age, and 92 were 1½ to 3 yrs of age. Approximately 33% were normal; the remaining 67% showed varying degrees of apparent genital developmental anomalies, specifically mispositioned and undersized scrota and ectopic testes, and this percentage held through all age groups. The sex ratio of fawns and fetuses was skewed towards males, significantly so for the 1996 fawn cohort and for the total of all fawns and fetuses in the study. Although possible causes of the genital anomalies, centering on endocrine disrupting pesticides, are discussed, no conclusions of cause and effect can be currently justified.

Key words : Genital abnormalities, White-tailed deer, Montana, Pesticides toxicity.

Introduction

This paper summarizes observations of unexpectedly high numbers of genital abnormalities in male white-tailed deer, *Odocoileus virginianus*, recorded at the Bitterroot Wildlife Rehabilitation Center in Ravalli County, Montana, USA. For the 5 year period beginning in January, 1996 and ending in March, 2000, approximately 2/3 of the male deer brought into the center had genital abnormalities of some degree, in contrast to no similar anomalies observed during the preceding 15 years. Although no cause is assigned, the influence of pesticides and other endocrine-disrupting compounds (EDC's) must be seriously considered in light of local herbicide spraying of roadsides and agricultural fields. In addition, seasonal long-range drift of herbicides and fungicides is likely to impact the study area during the passage of weather fronts, especially from areas of intensive cultivation to the south and west in Idaho, Oregon, and eastern Washington.

Many organic compounds have molecular structures that allow them to bind to

the nuclear receptor superfamily (Weatherman *et al.*, 1999). When this happens, disruption of the normal activity of a variety of hormones can occur. Examples are sex hormones, glucocorticoids, and vitamin D; some pesticides can also react with thyroid hormone and vitamin A receptors (Rolland, 2000). Among hundreds of pesticides in recent or current use, many are weak estrogenic or androgenic compounds that can act as agonists or antagonists to estrogen and androgen receptors (Colborn *et al.*, 1993, 1995; Gray *et al.*, 1999). Endocrine effects can also occur when pesticidal compounds interfere with biochemical reactions of synthesis, degradation, or conversion of endogenous hormones (Van Der Kraak *et al.*, 1998). Importantly, if exposure to one or more of these EDC's occurs in gestation during critical periods of organ development, a variety of developmental abnormalities can result (vom Saal *et al.*, 1992).

Although identifying a particular cause for observations in a *post hoc* data report such as this is virtually impossible, especially when the observations cannot be made until weeks or months have elapsed since the causative event,

we think it is important to present the findings for several reasons. Among them is the obvious possibility that the human population in the study area may be vulnerable to the same type of developmental problem. Also this is apparently the only report of widespread genital abnormalities in a population of wild ungulates.

Materials and Methods

The study area : Ravalli County, Montana, is in the far western sector of the state, approximately midway between the northern and southern state lines. The most prominent geographic feature is the Bitterroot Valley (BV), formed by north-flowing Bitterroot River, bounded on the west by the Bitterroot Mountain Range, with peaks to 3030 M, and on the east by the Sapphire Range, with peaks near 2700 M. Both ranges are forested, while the valley floor (under 1050 M) is agricultural and residential, with extensive riparian vegetation along the river and its tributaries. White-tailed deer, *Odocoileus virginianus*, are abundant on the valley floor. A major highway with heavy traffic runs north and south through the valley; another less used north-south highway parallels the first in the northern half of the valley, and a network of secondary roads serves homes and ranches throughout the BV. Deer-car collisions occur frequently on the highways and the secondary roads and account for a significant number of deer mortalities.

Collection of data : Since its beginning in 1980, the center has received many of the deer killed or injured along the valley roads, using the carcasses as food for carnivores undergoing rehabilitation. In addition, orphaned and injured fawns are often brought to the center. In the course of butchering the carcasses of road-killed animals or those whose injuries precluded their survival, it was noted that significant numbers of the male fawns born in 1995 had malformed and undersized scrota, often with ectopic testes positioned against the body wall just dorsal to the scrotum.

Beginning in spring, 1996, through early spring, 2000, the condition of the external genitalia of all male deer brought to the center was described and recorded. Scrotum length was

taken from the base of the scrotum on the left side to its most distal point. Abnormally small scrota in deer aged 1½ years or older were conservatively categorized as those under 45 mm, based on a normal testis length of 70–90 mm measured in 8 adults with normal testes. In 10 juvenile deer (3 months to one yr of age) with normal appearing genitalia, testis length was 45 mm or greater; therefore scrota less than 25 mm were conservatively considered abnormally short in this age class. Small scrota were often accompanied by aberrant location and alignment, and in the abnormal position of the testes. These types of anomalies were also recorded.

The year of birth of study animals was determined by examining the tooth eruption of each animal after methods outlined in Mosby (1963). With both year of birth and day of death (± 2 days) known, age determination was accurate to within one month. The number and age of accident-killed females was also recorded, although anatomical observations are not included in this report.

During the 15-year period from 1980 to 1994, a conservative estimate of 25 accident-killed, male white-tailed deer per year were processed in the rehabilitation center. Records of scrotum size and testes position were not kept during this period because genital abnormalities were not apparent.

Results and Discussion

Abnormalities in size and placement of scrota, and in the position of testes, are described and delineated in Tables 1, 2, and 3 for 254 deer in three age groups : fawns (3 months to 1 yr); juveniles (1 to 1½ yrs); and adults (1½ to 3 yrs). Differences in categories between the 3 tables arise from age and size differences in the animals. The pattern, however, is clear. Approximately 1/3 of the animals in each group had normal scrota with testes fully contained within them, when summed for the entire study period; the remaining 2/3 showed some degree of genital abnormality. Fig. 1 shows our observations by age class grouped for the entire study period, with status of the genitals combined

into 3 major categories. Fig. 2 and 3 illustrate the two most common abnormalities: the placement

of the right and left hemiscrotum along the midline, with the medial septum oriented

Table – 1 : Position of scrotum and testes in accident-killed white-tailed deer, age 3 mos. to one yr, Ravalli County, Montana.

Condition of scrotum and testes	Year			Total	%
	96-97	97-98	98-00		
Scrotum short, testes ectopic	8	10	1	19	14
One hemiscrotum underdeveloped, one testis ectopic	10	2	9	21	16
Right and left hemiscrota separated, both testes in scrotum	0	0	2	2	2
Scrotum rotated, septum perpendicular to midline, testes in scrotum	30	12	8	50	38
(Total with abnormalities)	(48)	(24)	(20)	(92)	(70)
Scrotum and testes size and placement normal	6	25	10	41	30
Total	54	49	30	133	100

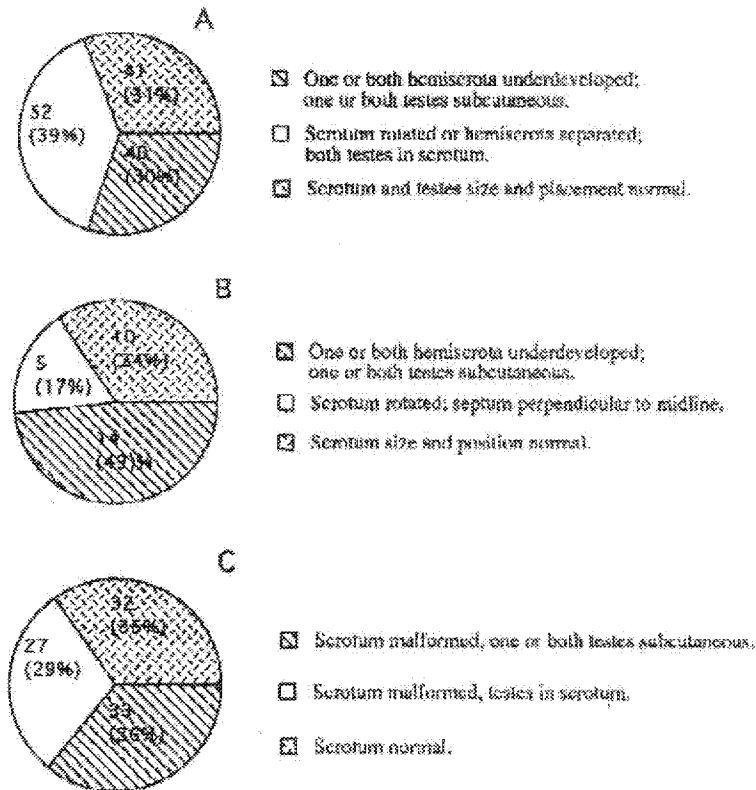


Fig. 1. Summary of developmental abnormalities in white-tailed deer, Ravalli Co., MT, 1995-2000. (A) age 3 months to 1 yr, (B) age 1-1½ yrs, (C) age 1½-3 yrs.

transversely; and the right hemiscrotum undersized and not enclosing the corresponding

testis. Dissection of several animals representative of this condition revealed that the

Table – 2 : Condition of scrotum and testes in accident-killed white-tailed deer, age 1 to 1½ yrs, Ravalli County, Montana.

Condition of scrotum and testes	Year			Total	%
	96-97	97-98	98-00		
Scrotum short, testes ectopic (against body wall)	7	1	0	8	28
One hemiscrotum underdeveloped, one testis ectopic	6	0	0	6	21
Scrotum rotated, septum perpendicular to midline	3	1	1	5	17
(Total with abnormalities)	(16)	(2)	(1)	(19)	(66)
Scrotum size and position normal	5	1	4	10	34
Total	21	3	5	29	100

left spermatic cord was routed anteriorly around an inguinal lymph node before it passed posteriorly through the inguinal canal, and that the point of attachment of both right and left gubernacula was variable, usually not at the apex of the scrotum.

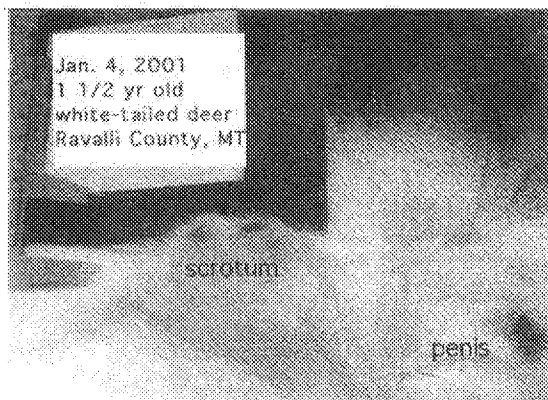


Fig. 2 Rotated scrotum on 1½ yr white-tailed old deer. The testes were ectopic, and the scrotal septum is perpendicular to the animal's midline.

Two by three contingency Tables were used to compare distributions of normal vs. abnormal for the three age classes by year in 1996-97, 6 fawns were normal in a total of 54, a highly significant difference (chi square=19.3, $p<0.001$) from fawns in 1997-98 (25 normal out of 49) and 1999-2000 (10 normal out of 30). The

distribution of normal vs. abnormal across all age classes for each of the 3 data-collection years did not indicate significant differences ($p > 0.05$). classes for each of the 3 data-collection years did not indicate significant differences ($p > 0.05$).

The sex ratio (Table 4) of fetuses and fawns combined, summed for the entire study period and analyzed by chi square test, using an expected ratio at birth of 100 females to 110 males, was skewed significantly toward males (130 females to 185 males, $p < 0.02$). The fetus ratio for the three years of data collection was 22 females to 35 males, while the fawn ratio was 108 to 150. The only individual cohort for which the ratio was significantly different was the fawn/1996-97 group (35 females to 55 males, $p<0.05$). The observations reported herein are clearly an experiment of nature, probably abetted by actions of human origin. Prior to 1995, there was no evidence of genital abnormalities in deer brought to the rehabilitation center. Moreover a biological and disease assessment of the white-tailed deer population on the nearby Lee Metcalf National Wildlife Refuge was conducted from December, 1990 to January, 1992. There is no mention of genital abnormalities among the 9 male deer examined in this careful study, although a number of other pathological

conditions were noted in the final report (O' Gara, 1992).

The abnormally small scrota in our study animals could result from failure of the testes to descend fully and thus expand the skin enclosing the pouch, or from a problem in formation of the scrotum *per se*. In many cases one or both testes were positioned against the body wall while the scrotum was empty. In other cases testes partially

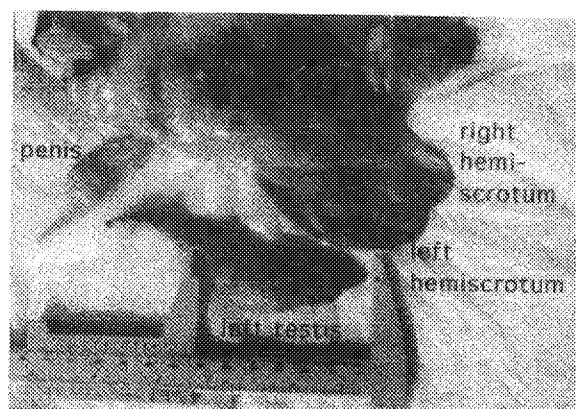


Fig. 3 Rotated scrotum on 1½ yr old white-tailed deer. The left testis was fully enclosed in the scrotum; the right testis was ectopic, against the body wall. Hair was shaved off for better viewing.

filled one or both hemiscrota, indicating that their descent was not complete, possibly because the attachment of the gubernaculum to the scrotum was abnormally positioned.

Assigning one or more causative factors to our observations would necessarily be a retrospective exercise lacking verifiable evidence and thus virtually impossible. Ankley and Giesy (1998) discussed the difficulty of bringing a weight of evidence argument to fruition in studying wildlife populations that are apparently impacted by xenobiotic chemicals. Nevertheless, the morphological anomalies described are characteristic of disrupted organogenesis during the gestational interval when male sex organs and accessory structures are developing (vom Saal *et al.*, 1992). If the anomalies did result from the action of xenobiotic chemicals, it would seem that their impact was most severe in the winter of 1995–96, since it was in the following months,

after the birth of the 1996 fawn crop, that 48 of the 54 fawns that came through the BWRC were abnormal. It may be of more than passing interest that in the same period, the sex ratio of fawns was significantly skewed toward males, indicating a differential mortality of female fawns or fetuses. It can be argued that the sex ratio of accident-killed fawns is biased toward males because of gender-based behaviour differences, but the trend is the same among the fetuses, and even though not significant, it supports the hypothesis of higher female mortality. It is most unfortunate that in the study area, and indeed the entire state of Montana, records of pesticide application by year, amount, and chemical species are not available; consequently we are unable to speculate on environmental impacts that may have been responsible for the especially severe problems observed in 1996–97.

Beard *et al.* (1999) exposed ewes in separate groups to the organochlorine pesticides lindane and pentachlorophenol ($1\text{mg}\times\text{kg}^{-1}$ body weight $\times\text{day}^{-1}$ mixed with feed) from 5 weeks before breeding to weaning of the lambs and continued the same dosage to the juvenile rams until puberty. They reported reproductive and endocrine effects and a slight increase in scrotum diameter (PCP-treated rams) but no developmental anomalies similar to those reported here. Gray *et al.* (1999) describe suprainguinal, ectopic testes in rats exposed perinatally to the fungicide vinclozolin, an antiandrogen. Ectopic tests, one or both, were a common observation in our study animals. We are unaware of published reports on cervid fetal ungulates exposed to EDC's, so neither we are able to make comparisons to closely related species nor can we speculate regarding the specific target of EDC's in our study animals. Nevertheless, the endocrine control of sex differentiation and development is highly conserved in mammals (Van Der Krak *et al.*, 1998), and we think that results from rodent research may offer an important clue to the anomalies of scrota and testes in the BV white-tailed deer.

There are multiple sources of xenobiotic chemicals in the BV, and multiple ways in which they can be dispersed in the environment. Locally, county weed control crews spray herbicides along roadsides and other "problem" areas. Residues of persistent herbicides on

roadside herbage and in alfalfa and grain fields are examples of sources of xenobiotic chemicals potentially available to the deer during much of the year. Tordon® (picloram, with or without 2,4-D, depending on the formulation), and 2, 4-D (2,4-dichlorophenoxyacetic acid) are

Table – 3 : Scrotum length and position of testes in accident-killed white-tailed deer, age 1½ to 3 yrs, Ravalli County, Montana.

Condition of scrotum and testes	Year				
	96-97	97-98	98-00	Total	%
Scrotum length ≤ 25 mm ^a	2	6	1	9	10
Scrotum length 26-45 mm ^a	7	2	4	13	14
Hemiscrota separated, length ≤ 25 mm ^a	0	0	1	1	1
One hemiscrotum not present ^a	2	1	1	4	4
Scrotum length ≥ 45 mm; one hemiscrotum underdeveloped ^a	2	2	1	5	5
Right and left hemiscrota separated, length ≥ 45 mm ^a . Left hemiscrotum anterior to right, septum perpendicular to midline, length ≥ 45 mm ^b	9	12	6	27	29
(Total abnormal) ^c	(22)	(23)	(14)	(59)	(63)
Scrotum size and position normal ^{b,d}	6	19	7	32	36
Totals	28	43	21	92	100

a. One or both testes ectopic (against body wall).

b. Both testes fully enclosed in scrotum.

c. All animals in this category were age 1½ to 1¾.

d. Category includes 2 animals age 2½ or greater.

Table – 4 : Sex ratio of accident-killed white-tailed deer fetuses and fawns, 1996-2000.

	Females	Males	Total	%F	%M	P ^a <
Fetus, 1997	5	9	14	35.7	64.3	0.37
Fetus, 1998	7	8	15	46.7	53.3	0.94
Fetus, 1999	10	18	28	35.7	64.3	0.21
Total fetus	22	35	57	38.6	61.4	0.17
Accident-killed fawns ^b						
1996-97	32	55	87	36.8	63.2	0.04
1997-98	46	61	107	43.0	57.0	0.34
1998-2000	30	34	64	46.9	53.1	0.91
Total a-K fawns	108	150	258	41.9	58.1	0.06
Total	130	185	315	41.3	58.7	0.02

a. p is probability based on chi square analysis, with expected ratio at birth of 100 females to 110 males.

b. About 2/3 of accident-killed fawns were from vehicle collisions; the remaining 1/3 were killed in miscellaneous accidents or euthanized following injuries incompatible with life in the wild.

commonly used. Farmers, orchardists, and ranchers spray a variety of pesticides, depending on the particular perceived needs of each; and the

U.S. Forest Service uses herbicides extensively within the national forest lands bordering the valley. Altogether, at least 16 different

herbicides, 3 insecticides, 4 fungicides, and one arachnicide have been used in the BV during the period of this research (Johnson, 1998, personal comm.).

Added to the local sources are agricultural chemicals that can be carried into the study area by storm winds from regions of intensive cultivation in the states of Washington, Oregon, and Idaho. Weather fronts typically bring strong SE to SW winds, which change to W and NW as the front passes. Potato fields in eastern Washington and southern Idaho, and grain fields in eastern portions of Washington and Oregon, are potential sources of herbicides and fungicides carried by these winds to the BV. Although many pesticides break down rapidly, others are more stable and persist in the fields where they were sprayed. They can be transported long distances when strong winds pick up pesticide-contaminated soil from unprotected fields, or when volatile species vaporize and become components of the atmosphere. Chernyak *et al.* (1996) reported evidence of a number of pesticides, some of which probably came from the Asian mainland, in the Bering and Chukchi Seas and associated air, ice, and fog layers; Wilkening *et al.* (2000) reported that wind borne persistent organic pesticides transit the Pacific Ocean from Asia episodically, mostly in April and May; Seba and Prospero (1971) found pp'-DDT and pp'-DDE in trade wind aerosols collected in Barbados, West Indies; and in a global sampling program, Simonovich and Hites (1995) described the worldwide distribution of 22 organochlorine compounds found in more than 200 tree bark samples. In March 1998, a rainwater sample collected by JAH near Stevensville, MT, and analyzed by Energy Laboratories, Inc., Billings, MT, tested positive for the fungicide chlorothalonil (tetrachloroisophthalonitrile), at 0.03 ppb (minimum). Below, we discuss this particular pesticide as a case study of a possible EDC impacting the BV white-tailed deer, albeit one among many possibilities.

Chlorothalonil is not used locally but is heavily used to control potato blight in both

Idaho and Washington. Although it is relatively nontoxic to mammals when given orally, it is highly toxic when inhaled and extremely toxic to fish, with a LC₅₀ of 10–76 ppb (Cox, 1997). A primary breakdown product, 4-hydroxytrichloroisophthalonitrile, is about 30 times more acutely toxic than its parent compound and does extensive damage to rats in chronic toxicity tests (World Health Organisation, 1996). It also meets an important criterion of estrogen-active compounds in possessing a phenolic hydroxyl group (Welshons *et al.*, 1999). Use of chlorothalonil in large amounts surged during summer and fall, 1994, when late blight fungus (*Phytophthora infestans*) spread into potato-growing areas in the West. Perhaps not coincidentally, abnormalities in the BV deer first appeared in fawns born the following spring. It is worth noting that air quality in the study area is not impacted by nearby polluting industries, so we have tentatively ruled out EDC's from local sources of that type.

The breeding season for white-tailed deer in the BV is November and early December. With a gestation period of 27–30 weeks, the critical time for sexual differentiation and formation of male genitalia would be approximately fetal age 8 weeks, thus late January into early March, a period during which local herbicide use is nil. Pesticide exposure during this period could come from residues applied locally earlier in the year, from long range drift as discussed above, or by an existing body burden. Although concentrations of EDC's drifting in might be extremely low, serum concentrations of such compounds sufficient to modify the actions of endogenous hormones are only in the pg/ml or ng/ml range (Welshons *et al.*, 1999; Rea *et al.*, 2000). Deer and other wild ungulates are uniquely vulnerable to xenobiotic chemicals carried on the wind, deposited on vegetation, or dissolved in snow and rain, because they depend on the vegetation for food and streams and ponds for drinking water, and because their exposure to the atmosphere is continuous. Also, the surface film of standing water can serve as a concentrator of persistent chlorinated pesticides (Seba and Corcoran, 1969;

Seba and Snedaker, 1995). Moreover, many of the pesticides of concern are lipophilic (chlorothalonil is said to be an exception, Exttoxnet, 1996) and can accumulate in fatty tissues; consequently even small exposure doses can build up in the bodies of ungulates over time. Nor can the potential for synergistic effects of exposure to two or more endocrine disruptors be ignored.

For these reasons, our efforts to understand the ultimate causes of the developmental anomalies in BV white-tailed deer include not just locally-use pesticides but others that may be borne on wind from more distant regions. Methods for measuring the transport and fate of EDC's by atmospheric vectors, and their subsequent incorporation into animal tissues, will, we hope, be the topic of future research into the phenomenon of aberrant sexual organ development reported herein.

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